ENGINEERED INTESTINAL TISSUE AND USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The invention is in the field of intestinal tissue models and their use in assays. Disclosed are methods of assessing the ability of a candidate therapeutic agent to reverse, reduce or prevent intestinal injury by a potential toxic agent using a three-dimensional, engineered, bioprinted, biological intestinal tissue model. Also disclosed are methods of assessing the effect of an agent on intestinal function, the method comprising contacting the agent with a three-dimensional, engineered, bioprinted, biological intestinal tissue model.

Background Art

[0002] The intestinal mucosa plays a crucial role in regulating absorption, first-pass metabolism, clearance, drug-drug interactions, and can be a site of drug induced toxicity. Current in vitro systems and preclinical models utilized in drug development do not adequately recapitulate the complexities of native human intestinal tissue, leading to low safety and efficacy predictability and attrition in drug development.

[0003] Current preclinical models are limited in their ability to capture the complexities and function of human intestinal tissue [1-5]. Systemic availability, diminished efficacy, and off target effects remain challenges to the successful prediction of candidate drugs and contribute to attrition in drug development. Many predictive challenges can be attributed to the lack of preclinical tools to model the complexities of intestinal function in vitro [1-3]. Oral delivery is the most common method for drug administration. The intestine plays a crucial role in the extent of absorption of orally administered drugs and first-pass metabolism. The intestine also serves as critical site of off target toxicity for compounds such as NSAIDS [4] and chemotherapeutic agents [5], and serves as a site of drug-drug interactions [2, 6]. Standard 2D systems lack the complexity to accurately model outcomes such as low bioavailability, the result of a combination of low permeability and interplay of metabolic enzymes with influx and efflux transporters in the intestinal epithelium [3]. The predominant in vitro models used to study intestinal bioavailability and toxicity include intestinal microsomes and 2D monolayers. Microsomes are a convenient tool for the initial assessment of metabolism, but cannot model cellular level outcomes.

[0004] 2D cell monolayer models lack native context of cell-cell and cell-matrix interactions and are phenotypically limited, while genetic disparity of animal models may not provide a high correlation with human outcomes [16]. Current in vitro intestinal models include 2D cell monolayer models of cell lines originating from colorectal and duodenal tumors (e.g., Caco-2, HT-29, HT29-18N2 and HuTu80). However, altered metabolism in tumor cells compared to normal tissue is a major disadvantage of these models. And, these tumor models do not represent features of native intestinal epithelium. The Caco-2 cell line is the most established cell model used to mimic passive transport and predict intestinal absorption. Limitations of the Caco-2 model include a lack of P-450 metabolizing enzyme expression and activity, lack of robust intestinal transporter expression and function, variation with passage number, and inconsistencies between clones in the line. Other 2D models include intestinal epithelial cells which, along with other cell lines, may have limited intestinal epithelial function due in part to their isolation from the other specialized epithelial cell types (ex: goblet cells, Paneth cells) as well as from the other supportive cell types present in the intestinal wall.

[0005] Limitations of commonly used cell lines have sparked the development of methods to use primary human intestinal cells. Monocultures of primary intestinal epithelial cells more closely resemble in vivo tissue but may have limited intestinal epithelial function in part to their isolation from the other supportive cell types present in the intestinal wall. In addition, testing in isolated epithelial monocultures prevents the ability to see effects on the interstitial and immune cells present in native tissue.

[0006] More complex 3D structures include intestinal segments and gut organoids derived from whole tissues or biopsies. The discovery of organoids to expand primary human intestinal cells [9, 10] or differentiate pluripotent stem cells [11] revealed another path to model the intestine in vitro. Organoids can be derived from all regions of the intestinal tract [12] and have been applied to many areas of intestinal research including organ development, disease modeling, and regenerative medicine [13, 14]. These structures suffer from low availability (from humans), limited viable lifespan in vitro, and may lack in vivo organ physiology. Notably, the closed lumens and the inward orientation of epithelia in intestinal organoids makes the apical surface relatively inaccessible for the direct stimulation they would normally experience in vivo, and makes organoids incompatible with most standard ADME/Tox assays.

[0007] Intestinal slices derived from human tissue can provide the correct cellular architecture and complexity as well as level of metabolic activity of native tissue. Intestinal slices, however, have limited viability ex vivo and only function for about 24 hours. Furthermore, these tissues are not compatible with cryopreservation, which limits their use to short term studies [15].

[0008] Animal models are frequently used to estimate compound bioavailability, however genetic differences can lead to disparity in expression of metabolic enzymes and transporters compared to humans which can result in poor prediction [2, 3, 16].

[0009] To overcome existing limitations of the current in vitro systems, a n automated bioprinting platform was utilized to develop a reproducible, highly cellular 3D primary human tissue model to recapitulate key aspects of the architecture of the native intestinal mucosa. Compared to standard 2D monolayer cultures, the 3D bioprinted intestinal tissue models create a more physiologically relevant environment, allowing for cells to establish cell-cell and cell-matrix interactions found in native tissue. The model, which incorporates a polarized intestinal epithelium supported by an interstitial tissue layer, is compatible with both histological and standard biochemical ADME/Tox readouts.

[0010] The 3D bioprinted intestinal tissues exhibit native-like layered architecture, including polarized epithelial morphology and physiological barrier function maintained for over two weeks in culture. The 3D bioprinted intestinal tissues express key P450 metabolic enzymes and transporters with similar endogenous levels compared to native intestine and demonstrate functional activity of both